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PRETRAINING EXPOSURE TO PHYSIOLOGICALLY PATTERNED ELECTROMAGNETIC STIMULATION ATTENUATES FEAR-CONDITIONED ANALGESIA

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The effect of weak electromagnetic stimulation on the emergence of conditioned analgesia was examined in the adult rat. Subjects were conditioned to associate a continuous 20 kHz ultrasonic tone (CS) with 0.2 mA footshock (UCS^-) over five successive days. For 30 min either before or after conditioning sessions, rats were exposed to sham or pulsed (primed burst potentiation) magnetic fields (500 nT). At the end of the conditioning phase, all animals were evaluated for anticipatory analgesia following CS presentation using a hotplate analgesiometer. Data analysis suggested a statistically significant attenuation of fear-conditioned analgesia in rats exposed to electromagnetic stimulation prior to conditioning, whereas post-conditioning exposure potentiated the reduction in pain sensitivity compared to baseline measures. The present results suggest that the emergence of fear-conditioned responses is sensitive to whole body exposure to a magnetic field pattern that has been shown to induce long-term potentiation in hippocampal slices.

Keywords: Analgesia; conditioned fear; footshock; electromagnetic fields; rat; ultrasound

INTRODUCTION

Conditioned fear is a learned response to stimuli that precede pain or danger. A consequence of this form of learning is that behavioral reactions to previously injurious situations may be characterized by a suppression of

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competing responses (Walters, 1994). Because defensive behaviors and nociceptive responding are incompatible activities, organisms have evolved antinociceptive systems. They enable an individual to engage in optimal defensive behaviors without an inhibiting motivation to respond to a present injury.

In fear conditioning, a rat is placed in a novel environment (consisting of numerous contextual stimuli) that is paired with noxious stimulation (*i.e.*, peripheral shock). Behavioral responses to shock are characterized by activity bursts, including rapid locomotion and startle-like responses, defecation, and crypsis (freezing). Learning is inferred as alterations in the strength of synaptic connections within pre-existing brain pathways (Sommerhoff, 1974) and is measured by changes in an animal's response patterns.

The consolidation of information through learning ensures the emergence of new behaviors or alterations in existing behavior that will aid the organism in adapting to environmental or physiological challenges. Considerable evidence suggests that memory consolidation progresses through different stages, each of which is sensitive to forced manipulation (Thomas and Persinger, 1997; Cahill and McGaugh, 1998). The effect of post-training electromagnetic (EM) stimulation on spatial learning has been shown (Thomas and Persinger, 1997) and implies that the consolidation and representation of experience may be sensitive to exogenous stimulation.

Rose and coworkers (1988) developed a pattern of stimulation, referred to as primed burst potentiation (PB), that was shown to induce long-lasting increases in hippocamal (CA1) synaptic activity following afferent stimulation (Fig. 1). When this pulse train was delivered prior to an LTP (long-term potentiation)-inducing stimulus, the effect was not additive. PB inhibited the effect of the LTP-inducing stimulus upon the production of LTP.

Antagonism at N-methyl-D-aspartate (NMDA) receptor sites also inhibited PB, suggesting that the two processes shared an underlying mechanism. Later experiments confirmed that the induction of LTP by tetanic stimulation was inhibited by prior activation of N-methyl-D-aspartate (NMDA) receptors at those same synapses. Evidence suggested that NMDA receptor activity, which preceded the LTP-inducing stimulus produced a subtle synaptic change which effectively raised the stimulation threshold for LTP (Abraham and Bear, 1996).

The present study was designed to discern the behavioral effects of PB stimulation, presented as whole-body exposure to an EM field, upon the associative learning process during a fear-conditioning paradigm. We reasoned that if this pattern of magnetic field functionally simulated the effects of PB, then field exposure, when delivered prior to conditioning

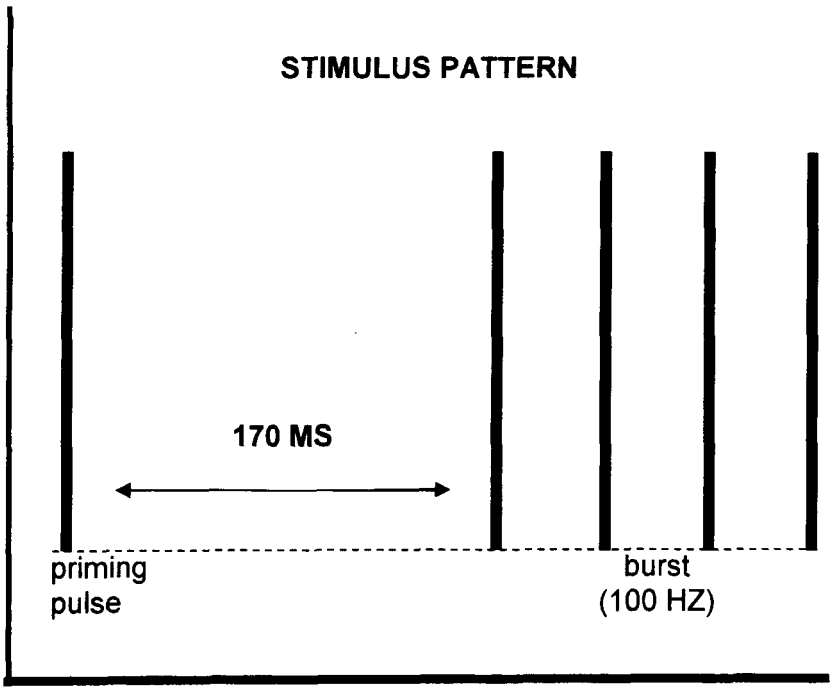

FIGURE 1 Pattern of whole-body electromagnetic stimulation (Adapted from Rose *et al.*, 1988).

episodes, should interfere with the emergence of fear-conditioned responses (hypoalgesia) after repeated, signaled delivery of noxious stimulation.

MATERIALS AND METHOD

In two blocks of experiments, 43 female Wistar rats (Charles River, Quebec) age 8 to 11 months served as subjects. Animals has been housed three per cage in a climate-controlled colony room (20–21°C); food and water were available *ad libitum*. The light-dark cycle was set at 12:12; the dark cycle began at 1930 hrs. All testing was conducted during the mid-light cycle.

All subjects were evaluated using a hotplate analgesiometer for baseline nociceptive thresholds five days before conditioning began. During the conditioning phase (5 days), training sessions consisted of each subject being placed in a $28 \times 20 \times 20$ cm Plexiglass chamber with a raised floor composed of steel bars. The intensity of electric current through the bars was

controlled by an A-615-C Master Shocker (Lafayette Instruments) and was set at 0.2 mA for all sessions.

A continuous 20 kHz ultrasonic tone (60 db) was generated through a small speaker mounted behind a circular opening in the chamber for the duration of the training session (1 min) and served as a conditioned stimulus (CS) to signal the delivery of footshock (UCS). A brief shock (1 sec) was then delivered 30 sec after the beginning of a session. Prior to the conditioning phase, subjects had been randomly assigned to one of two EM field exposure groups, either for 30 min after each conditioning session. Sham field subjects were employed as the reference group.

During magnetic field exposure, 12 cages, each containing one rat, were placed in aluminum racks (6 cages per rack). The dimensions of the perimeter of each rack was 112×125 cm. It was wrapped by 72 turns of 30 American wire gauge (AWG) resulting in a width of 13 cm. The experimental field was generated by a custom designed digital-to-analogue converter through one of the racks and was patterned to simulate primed burst potentiation (Fig. 1). The pattern was created by a simple conversion of a series of numbers between 125 and 250 to voltage output; all values were of positive polarity. The duration of each of the 224 successive temporal points that constituted the pattern were programmable.

In the present study, the pixel duration was 1 msec; this meant that each voltage option lasted for 1 msec + 190 μsec (response latency of the computer). The duration of one cycle of the program was 266 msec, resulting in 3.76 presentations per second. The field strength generated through the coil was measured by a Metex 3800 multimeter and magnetic sensor probe (Electric Field Measurements, Stockbridge, MA) and varied between .3 μT and .5 μT (300 nT to 500 nT).

At the end of the conditioning phase subjects were again tested for nociceptive thresholds following a 2 min exposure to the conditioning apparatus. During this phase the ultrasound was presented for the first minute but no shock was delivered. All data were analyzed using SPSS software on a VAX 4000 computer.

RESULTS

The means (+/− SEM) for post-conditioned nociceptive measures are shown in Table I. A two-way analysis of variance indicated a significant interaction between field and time of exposure [$F_{(3,42)} = 10.03$, $p < .001$, $\omega^2 = 3\%$].

TABLE I Means and standard errors of the mean (SEM) for hotplate latencies (sec) after the conditioning phase

Field/Exposure time	*N*	*Mean*	*SEM*
Sham/pre	11	24.70	1.56
Pulse/pre	11	17.24	1.06
Sham/post	10	17.53	0.90
Pulse/post	11	22.52	1.01

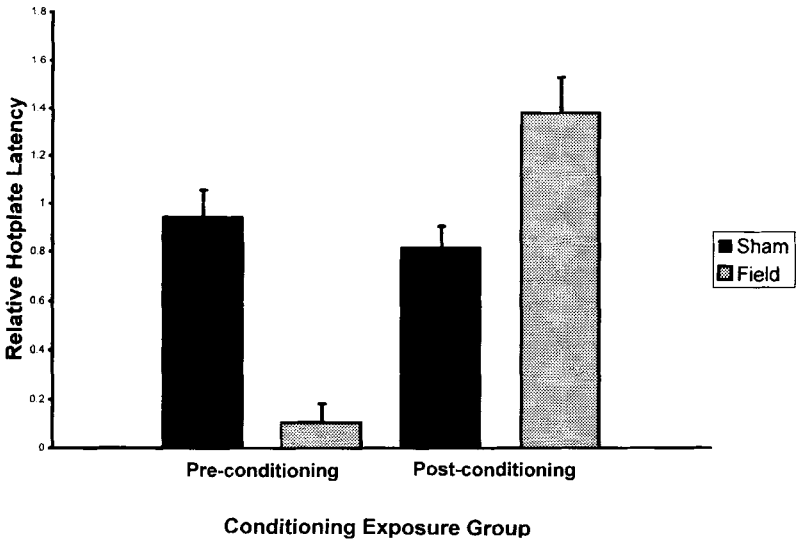

FIGURE 2 Means and standard errors of the mean for hotplate response latencies as a function of experimental treatment.

These results did not accomodate pre-conditioning values. Therefore, relative scores were computed for each subject and analyzed. A significant main effect for time of field exposure [$F_{(1,42)} = 53.40$, $p < .001$, $\varepsilon^2 = 37\%$] and a significant interaction between field and time [$F_{(3,42)} = 34.48$, $p < .001$, $\varepsilon^2 = 39\%$] were noted. *Post hoc* analysis (Fig. 2) indicated subjects that received EM stimulation following each conditioning session showed a significantly ($p < .05$) higher relative hotplate latency (more time spent on the hotplate compared to baseline) than the sham field groups, all of which did not differ significantly from each other. The relative hotplate latency for the pre-trained, stimulated rats, differed significantly from all groups.

In addition to reducing shock-induced analgesia, post-conditioning EM exposure was also shown to enhance responses following exposure to the conditioning apparatus and to the signaled delivery of footshock. The most qualitatively conspicuous behavior was the almost immediate emergence

(after one pairing trial) of freezing, defecation and urination. Marked increases in post-shock locomotor activity in the majority of subjects receiving post-conditioning EM stimulation was also conspicuous.

DISCUSSION

The influence of electromagnetic fields upon cognitive processes has been the subject of considerable investigation (Richards *et al.*, 1993; Persinger *et al.*, 1994; Belisheva *et al.*, 1995; Nikol'skaya *et al.*, 1996). However, the exact mechanism of neuronal reactions to such stimulation still remains unclear. The behavioral consequences of exposure of subjects to low-intensity magnetic fields at variable points during behavioral paradigms has not been investigated systematically. Thomas and Persinger (1997) has recently shown that post-training exposure to a weak, disymetrical-patterned, magnetic field affected motivation but not proficiency during spatial learning in rats. Kavaliers and coworkers (1993) also reported an enhanced acquisition of spatial learning in rodents exposed to a magnetic field which simulated opioid a antagonism.

To pursue the hypothesis that EM stimulation may raise the threshold for LTP and for subsequent learning to occur, the present study involved daily exposures to a pulsed field that was modeled after primed burst potentiation. The results of this experiment indicated a reduction in pain sensitivity following a series of conditioning episodes. In light of previous findings demonstrating the effects of this LTP-inducing pulse on hippocampal synapses, repeated exposure to this stimulus pattern before conditioning trials may have raised the stimulus threshold at which the cellular processes underlying associative learning occurred. This change could also account for not only the quantitative decrease in analgesia, but also the reduction of other (qualitative) conditioned responses to the footshock environment (*i.e.*, freezing and incontinence). The persistence of the rapid motor activity during conditioning sessions and following footshock indicated that these qualitative changes were not due to nonspecific effects.

Contextual fear-conditioning is believed to be mediated by the hippocampal formation. Despite claims that the hippocampus does not play a significant role in Pavlovian conditioning, many researchers contend that the hippocampal formation is pivotal in conditioning to context, which would consist of multimodal inputs including tactile, spatial and visual stimuli (Maren *et al.*, 1998). Maren and Fanselow (1997) reported lesions to the dorsal hippopcampus (DH) and its primary afferent projection systems, (fimbria/fornix (FX) and entorhinal cortex (EC), have been shown to produce deficits in fear-conditioning to contextual stimuli. These investiga-

tors found that lesions restricted to these areas of brain resulted in: 1) a marked decrease in freezing behavior in response to contextual cues, and 2) increases in locomotor activity during conditioning sessions.

Although some questions still remain concerning the exact role of the FX and EC in the relay of contextual input to the hippocampus, Maren *et al.* (1994a, b) established that stimuli which facilitate theta rhythm (associated with synaptic plasticity in EC-DH connections) also enhanced conditioned fear to contextual cues. By using a pattern of stimulation modelled after hippocampal theta rhythm and complex spike discharge (Rose *et al.*, 1988), we suggest that the threshold for contextual learning was shifted by prior activity (Abraham and Bear, 1996) so that strong stimulation was less likely to produce LTP within the EC-DH pathway.

The present results also suggest a decrease in LTP-inducing sensory input to the DH which is critical for the processing of contextual information (McNish *et al.*, 1997). However, decreases in freezing and conditioned analgesia in response to both discrete and contextual stimuli have been reported following lesions of the amygdala and periaqueductal gray (PAG) (Helmstetter, 1992; Kim *et al.*, 1993). Information related to auditory conditioning is relayed from the medial geniculate nucleus (MGN) to the lateral amygdala *via* activation of NMDA receptors (McKernan and Shinnick-Gallagher, 1997). This pathway has been shown to support neural plasticity in the form of LTP and the acquisition of auditory-induced conditioned fear is paralleled by LTP-like processes (Rogan *et al.*, 1997). Indirect projections from the lateral nucleus to the central amygdala (Davis *et al.*, 1994) would play a critical role in the expression of fear-related responses to conditioned stimuli *via* activation of the PAG (Bellgowan and Helmstetter, 1998). Interference with associative plasticity within this pathway (not unlike that within EC-DH connections) may also disrupt the expression of conditional responses.

Thus, the evidence obtained from the present experiment demonstrates a differential sensitivity of rats to the appropriate pattern of magnetic field during a learning paradigm. The effect was obtained with exposure intensities of only 500 nT. Such EM stimulation may have a more powerful role than now suspected upon the normal course of cognitive activity.

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